CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-227

PHARMACOLOGY REVIEW

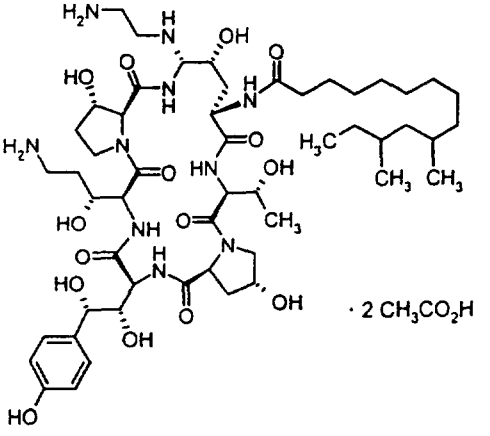
KEY WORDS: CANCIDAS™, Caspofungin acetate, caspofungin, MK-0991, MK0991, L-743872, intravenous, antifungal, glucan synthesis inhibitor, invasive aspergillosis, histamine release, hyperemia, liver toxicity, injection site irritation, embryotoxic, incomplete ossification, cervical rib, resorptions.

Reviewer Name: Owen McMaster
Division Name: Division of Special Pathogen and Immunologic Drug Products
HFD-590
Review Completion Date: January 16, 2001
Related IND: [redacted]
Serial number: 000
Date of Submission: July, 28, 2000
Information to sponsor: Yes
Sponsor: Merck and Co.
P.O. Box 4
West Point PA 19486

Manufacturer of the drug substance: Merck and Company

Drug: CANCIDAS™
Code Names: MK-0991, L-743872
Generic Name: caspofungin acetate
Trade Name: CANCIDAS™
Chemical Name: (4*R*.5*S*)-5-[(2-aminoethyl)amino]-N^2-(10,12-dimethyl-1-oxotetradecyl)-4-hydroxy-L-ornithyl-L-threonyl-trans-4-hydroxy-L-prolyl-(S)-4-hydroxy-4-(4-hydroxyphenyl)-L-threonyl-*threo*-3-hydroxy-L-ornithyl-*trans*-3-hydroxy-L-proline cyclic(6→1)-peptide diacetate salt.

CAS Registry Number: 179463-17-3
Molecular Formula: $C_{52}H_{88}N_{10}O_{15}\bullet 2C_2H_4O_2$
Molecular Weight: 1213.42
Structure:



Relevant INDs/NDAs/DMFs: [redacted]
Drug Class: Echinocandin

Indication: Treatment of invasive aspergillosis for patients who are refractory to, or intolerant of, other therapies.

Clinical formulation: Caspofungin for injection contains caspofungin acetate, (active ingredient, 46.6 mg/mL) sucrose NF[redacted] mannitol,USP[redacted] [redacted] acetic acid USP[redacted] sodium hydroxide, NF[redacted] [redacted] and water for injection[redacted]

Route of administration: Intravenous

Previous clinical experience: None

Introduction

Caspofungin is a semisynthetic lipopeptide (echinocandin) compound[redacted] Caspofungin is the first of a new class of antifungal agents that inhibit the synthesis of β(1,3-D-glucan, an integral component of the fungal cell wall. Patients will be treated with a loading dose of 70-mg caspofungin, followed by a 50-mg daily dose. The mean $AUC_{(0\text{-}24h)}$ values for patients with candida esophagitis treated repeatedly with caspofungin was 94 μg.hr/ml on day 1 and 167 μg.h/ml on day 14.

Toxicology Studies Summary:

A-1. Acute intravenous and subcutaneous toxicity studies in mice and rats. TT # 94-2887, TT#94-2888, TT#94-2889 and TT # 94-2890
A-2. Exploratory 48-hour acute intravenous toxicity study in male mice. TT # 95-2731
A-3. Exploratory acute oral toxicity study in mice. TT #97-2557
A-4. Acute intravenous toxicity study in rats TT #97-2502
A-5 Exploratory acute intravenous toxicity study in rabbits TT # 93-037-0
A-6 Exploratory 15-day intravenous toxicity study in rats TT # 93-037-0
A-7 Five-week intravenous toxicity study in rats TT # 94-637-0
A-8 Five-week intravenous toxicity study in monkeys. TT # 94-638-0
A-9 Fourteen-week intravenous toxicity study in monkeys TT # 95-613-0
A-10 Fourteen-week intravenous toxicity study in rats TT # 95-612-0
A-11 Five-week intravenous toxicity study in monkeys. TT # 96-073-0
A-12 Twenty-seven-week intravenous toxicity study in rats. TT # 97-120-0
A-13 Twenty-seven-week intravenous toxicity study in monkeys. TT # 97-121-0
A-14 Assay for chromosomal aberrations in mouse bone marrow. TT # 95-8713
B-1 Intravenous fertility study in male rats. TT97-714-0
B-2 Intravenous fertility study in female rats. TT#96-735-0
C1 Intravenous range-finding reproduction study in female rats. TT#96-730-5
C2 Intravenous developmental toxicity study in rats. TT#96-736-0
C3 Intravenous developmental toxicity study in rats-Post-weaning evaluation.

TT#96-704-0

C4 Intravenous Range-finding study in non-pregnant rabbits. TT#96-731-6
C-5 Intravenous Range-finding study in pregnant rabbits. TT#96-731-5
C-6 Intravenous developmental toxicity study in rabbits. TT#96-731-0
C-7 Intravenous toxicokinetic study in pregnant rabbits. TT#97-719-0
C-8 Intravenous toxicokinetic study in pregnant and lactating rats. TT#97-718-0
D-1 Microbial Mutagenesis Assay TT#94-8053, TT#94-8054, TT#95-8000, TT-8009
D-2 Microbial Mutagenesis Assay TT#96-8078, TT#96-8080, TT#97-8003
D-3 *In vitro* Alkaline elution/Rat hepatocyte Assay. TT#94-8244 and TT#94-8245
D-4 *In vitro* Alkaline elution/Rat hepatocyte Assay. TT#96-8313
D-5 Assay for chromosomal aberrations in vitro, in Chinese hamster ovary cells TT#94-8677, TT#94-8791
D-6 Assay for chromosomal aberrations in vitro, in Chinese hamster ovary cells TT#96-8724, TT#96-8732
D-7 V-79 Mammalian cell mutagenesis Assay. TT#95-8507, TT#95-8508, TT#96-8502, TT#96-8504
D-8 Assay for chromosomal aberrations in mouse bone marrow, TT#95-8713

Toxicology Studies Review

Report A-1: MRL Nonclinical report: L-743872:
Study Title: Acute and subcutaneous toxicity studies in mice and rats.
Study Nos: TT#94-2887, TT#94-2888, TT#94-2889, TT#94-2890
NDA 21-227, Vol # 3, and page # A55:
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: October 17, 1994
GLP compliance: Yes
QA- Report: Yes

L-743,872 was prepared as a 0.2 percent solution in 0.9 % saline. Female animals were treated as described below, and observed for 14 days.

In study TT#94-2887, mice, Crl:CD-1®(ICR)BR strain, were injected intravenously with L-743,872 at 12.5 (3 mice), 25 (one mouse), and 50 mg/kg (one mouse)., While no deaths occurred at the 12.5 mg/kg dose, the mice given 25 or 50 mg/kg, died. The minimum lethal intravenous dose in mice was determined to be 25 mg/kg, which is equivalent to a human dose of 2 mg/kg (or 120 mg for a 60kg patient).

In study TT#94-2888 mice, Crl:CD-1®(ICR)BR strain, were injected subcutaneously with L-743,872 at 50 (1 mouse), 100 (three mice), 200 (3 mice) and 400 mg/kg (one mouse). Two of the three mice given 200 mg/kg died, as did the mouse treated at 400 mg/kg. The minimum lethal subcutaneous dose was determined to be 200 mg/kg, which is equivalent to a human dose of 16.2 mg/kg (a dose of 972 mg for a 60kg patient).

In study TT#94-2889 rats, Crl:CD-1®(SD)BR strain, were injected intravenously with L-743,872 at 50 (1 rat), or 25 mg/kg (three rats). The rat given 50 mg/kg died. The minimum lethal

intravenous dose was determined to be 50 mg/kg, which is equivalent to a human dose of 8.35 mg/kg (a dose of 501 mg for a 60kg patient).

In study TT#94-2890 rats, Crl:CD-1®(SD)BR strain, were injected subcutaneously with L-743,872 at 25 (1 rat), 50 (1 rat), 100 (3 rats), 200 (1 rat) or 400 mg/kg (1 rat). The rat given 200 or 400 mg/kg died. The minimum lethal subcutaneous dose was determined to be 200 mg/kg, which is equivalent to a human dose of 32 mg/kg (a dose of 1920 mg) for a 60 kg patient).

Common clinical signs included tremors, decreased activity and bradypnea. Discoloration/scabbing/abscess of the injection sites was also observed. In the subcutaneous rat study animals were observed with swollen muzzles and/or paws and with reddened ears, paws and tail.

MRL Nonclinical report: A-2

In the present summarized study, (L-743872: Exploratory 48-hour acute intravenous toxicity study in male mice TT # 95-2731), male Crl:CD-1®(ICR)BR mice were injected intravenously with L-743,872 for dose selection for an in vivo cytogenicity study. Doses of 16, 20, 25 and 31.25 mg/kg were used. Five of five mice died after receiving the 31.25 mg/kg dose and one of five died after receiving the 25 mg/kg dose. Thus, the minimum lethal dose for male Crl:CD-1®(ICR)BR mice was determined to be 25 mg/kg, which is equivalent to a human dose of 2 mg/kg or 120 mg for a 60kg patient.

MRL Nonclinical report: A-3
Study Title: L-743872: Exploratory acute oral toxicity study in mice.
Study Nos: TT#96-2757
Document location: NDA 21-227, Vol # 3
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: December 10, 1996
GLP compliance: No
Drug lot number: L-743872-003M011

Three female Crl:CD-1®(ICR)BR mice were treated by oral gavage with caspofungin (a 6 % suspension in 0.5 % aqueous methylcellulose) at 2000 mg/kg and observed for 7 days. Initial findings included decreased activity, bradypnea, ptosis and ataxia. These began 4 hours post-dose and lasted until day 2. Animals returned to normal on day 2.

No deaths were recorded and the minium lethal oral dose was determined to be greater than 2000 mg/kg . This is equivalent to a human oral dose of 162 mg/kg or 9720 mg for a 60-kg patient.

MRL Nonclinical report: A-4
Study Title: L-743872: Acute intravenous toxicity study in rats.
Study Nos: TT#97-2502
Document location: NDA 21-227, Vol # 3
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: January 14, 1997
GLP compliance: Yes
Drug lot number: L-743872-012P001 (0991 HLS 003A001)

Groups of female Crl:CD-1®(ICR)BR rats were treated intravenously with caspofungin (as a 0.25 % solution in 0.9 % sterile saline) at 25 (three animals) or 50 mg/kg (one animal). Three animals were injected with vehicle and served as controls. Animals were observed for 14 days following injection.

At 50 mg/kg, caspofungin produced clonic convulsions and bradypnea within one minute and eventually death. At 25 mg/kg, animals showed decreased activity, bradypnea, ataxia, sternal recumbency, redness (feet and ears), and swelling (muzzle, ears and feet) beginning as soon as one minute after dosing and lasting as long as throughout day 1. Purple discoloration of the tail injection site was also noted on days one and two.

The minimum lethal intravenous dose was determined to be 50 mg/kg in rats, a dose which is equivalent to a human dose of 8.4 mg/kg or 504 mg for a 60-kg patient.

MRL Nonclinical report: A-5
Study Title: Memo to Christine Huber from KimTrick: L-743872: Exploratory acute intravenous toxicity study in rabbits.
Study Nos: TT#95-2573
Document location: NDA 21-227, Vol # 3
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: 1995
GLP compliance: No
Drug lot number: L-743872-003M (Lot #6)

New Zealand white rabbits were treated intravenously with caspofungin at 8 (1 animal/sex), 12 (1 animal/sex) or 16 mg/kg (1 male) and observed for 4 to 5 days.

At 16 mg/kg, caspofungin produced tremors, bradypnea, lateral recumbency, cyanosis and vascular dilation within 5 minutes of injection. The animal died about 30minutes after injection. No physical signs seen in the two lower doses were judged to be treatment related.

The minimum lethal dose for caspofungin in New Zealand White rabbits was 16 mg/kg, equivalent to a human dose of 5.1 mg/kg or 307 mg for a 60-kg patient.

Summary and Conclusions

Acute toxicity studies conducted in mice, rats and rabbits, showed that the minimum lethal dose of caspofungin ranged from 2-8.4 mg/kg (equivalent human doses, based on body surface area conversions). The maximum nonlethal doses ranged from 1 mg/kg (mice) to 3.9 mg/kg in rabbits and 4.2 mg/kg in rats. Mice were four times more sensitive to caspofungin than rats and rabbits, and were not used for the repeat dose studies.

Repeat dose studies

MRL Nonclinical report: A-6
Study Title: L-743872: Exploratory 15-day intravenous toxicity study in rats.
Study Nos: TT#93-037-0
Document location: NDA 21-227, Vol # 3
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: 1993
GLP compliance: No
Drug lot number: L-743872-001H003

Groups of Crl:CD®(SD)BR rats, 5/sex/dose group, were treated intravenously with caspofungin at 0 (control), 2 or 5 mg/kg/day for 14 days. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, organ weights, necropsy findings, and histology.

In this preliminary study, there was no mortality. Significant findings in the low dose included transient redness of the ears and nose and swelling of the nose. Additional signs seen in the high dose included sluggish movement or ataxia, and sternal or lateral recumbency. Purple discoloration of the tail was observed intermittently throughout the study. These signs were not seen after day 5. No other significant drug related effects were reported.

MRL Nonclinical report: A-7
Study Title: L-743872: Five week intravenous toxicity study in rats.
Study Nos: TT#94-637-0
Document location: NDA 21-227, Vol # 3
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: 1994
GLP compliance: Yes
Drug lot number: L-743872-003M batch #006

Groups of Crl:CD®(SD)BR rats, 15/sex/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2 or 5 mg/kg/day for one month. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings, and histology. Plasma was obtained on dosing day 28 for plasma drug level determinations.

Significant findings at the high dose included transient redness of the ears and nose and swelling of the nose. Additional signs seen in the high dose included sluggish movement or ataxia, and sternal or lateral recumbency. Purple discoloration of the tail was observed intermittently throughout the study and a few progressed to crust formation, grayish coloration and/or skin necrosis. Except for the changes at the injection site, these signs were not seen after day 7. No other significant drug related effects were reported.

The pharmacokinetics parameters are shown in Table 1, below. Exposure to caspofungin, as measured by C_{max} and $AUC_{(2-24h)}$, was slightly greater than dose proportional. There were no gender-related differences in pharmacokinetics parameters when caspofungin was administered to these rats.

Table 1. Mean Plasma Pharmacokinetics parameters of caspofungin in rats on day 28.

Caspofungin dose (mg/kg/)	Cmax (μg/ml)	AUC(2-24h)
0.5	2.7	20.8
2	12.8	122
5	29.5	176

Comment: The first measurement of plasma drug levels was performed on blood drawn 2 hours after the intravenous dose. C_{max} is therefore not a true indication of the maximum plasma concentration achieved and the $AUC_{(2-24h)}$ is an underestimation of $AUC_{(0-24h)}$.

Table 2. Mean Liver levels of caspofungin in rats on day 28.

Caspofungin dose (mg/kg)	Liver drug level (μg/g)
0.5	21
2	70
5	172

Pharmacokinetics of caspofungin in the liver (Table 2) were slightly less than dose proportional. There were no sex-related differences in liver caspofungin levels.

Conclusion

Caspofungin injection in rats produces signs of histamine release and injection site damage. The exposure to caspofungin increases with increasing dose in the plasma and the liver, where the increase is slightly more and slightly less than dose proportional, respectively.

MRL Nonclinical report: A-8
Study Title: L-743872: Five week intravenous toxicity study in monkeys.
Study Nos: TT#94-638-0
Document location: NDA 21-227, Vol # 4 page A-579
Conducting laboratory and location: [redacted]
Date of study initiation: 24 October, 1994
GLP compliance: Yes
Drug lot number: L-743872-003M (batch #6)

Groups of rhesus monkeys (*Macaca mulatta)*, 4/sex/dose group, were treated intravenously with caspofungin at 0 (control), 2, 5 or 8 mg/kg/day for five weeks. Infusion was performed via the saphenous veins of the right and left legs alternatively and the total dose was delivered in twenty minutes (0.4 to 0.6 ml/min). Dose volume was 4 ml/kg. Later in the study, due to difficulties with injections via the saphenous veins, injections were performed via cephalic veins, and if necessary, via manual or pedal veins. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings, and histology. Ophthalmic examinations were conducted on all monkeys pretest and during week 4. Plasma drug levels were measured on days 1 and 28 and liver drug levels were determined at necropsy.

In some high dose animals (beginning after a few days of treatment) a portion of the saphenous vein did not dilate after proximal compression, making visualization difficult. This affected 5 and 7 of 8 in the mid- and high- dose animals respectively. This also occurred in one instance in a low dose animal (day 15). Animals developed progressive hardening of the area along the saphenous vein and, in half of the animals, this change persisted until the end of the study. By the end of the study, cephalic, manual or pedal veins were being used since injection at the saphenous vein injection site was difficult or impossible. Transient purplish areas were noted at injection sites in all groups. Necrosis of the skin overlying veins was noted in one female from the mid dose and two males from the high dose group.

Caspofungin administration at 5 and 8 mg/kg produced several changes in the liver. Increases in AST and ALT levels are shown on Table 3, below. Foci of subcapsular necrosis were seen in both sexes, with lesions scattered throughout the subcapsular area, ranging from single-cell to muticellular necrotic foci, with polymorphonuclear and/or mononuclear cell infiltrate. There was also an increase in total bilirubin (50% and 100%, respectively) at 5 and 8 mg/kg (at week 4).

Table 3. Effect of caspofungin on AST and ALT levels following 2 or 4 weeks of dosing.

Dose week	Increase in AST (%)		Increase in ALT (%)	
	5 mg/kg	8 mg/kg	5 mg/kg	8 mg/kg
2	37	80	112	191
4	21	69	83	177

Table 4. Incidence of multifocal subcapsular necrosis of the liver in monkeys treated for 5 weeks with caspofungin.

	Control	2 mg/kg	5 mg/kg	8 mg/kg
Females (n=4)	0	0	2	3
Males (n=4)	0	0	0	1

Caspofungin injection into monkeys for 5 weeks was associated with difficulty with venous dilatation at all doses so no NOAEL could be established from this study. At higher doses, caspofungin administration was associated with liver damage, characterized by increased AST and ALT levels, increased bilirubin levels, as well as subcapsular necrosis and scarring (Table 4). Injection site damage was seen at all doses. Injection site damage in dosed animals consisted of thickening and discoloration of veins and perivenous tissue with hemorrhage in adjacent tissue. Findings were most severe in high dose animals. In addition, venous thrombosis was detected in all animals in the mid and high dose groups.

MRL Nonclinical report: A-9
Study Title: L-743872: Fourteen-week intravenous toxicity study in monkeys.
Study Nos: TT#95-613-0
Document location: NDA 21-227, Vol # 5 page A-861
Conducting laboratory and location: [redacted]
Date of study initiation: 28 April, 1995
GLP compliance: Yes
Drug lot number: L-743872-003M (batch #11)

Groups of rhesus monkeys *Macaca mulatta*, 4/sex/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2 or 5 mg/kg/day for fourteen weeks. Infusion was performed via the saphenous veins of the right and left legs alternatively and the total dose was delivered in twenty minutes (1-1.5 ml/min). Dose volume was 8 ml/kg and was followed by a 5-ml flush of saline. Later in the study, due to difficulties with injections via the saphenous veins, injections were performed via cephalic veins, and if necessary, via manual or pedal veins. Records were kept of mortality, clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings, and histology. Ophthalmic examinations were conducted on all monkeys pretest and during week 13. Plasma drug levels were measured in weeks 4, 8, and 12.

In some high dose animals (beginning in week 3) a portion of the saphenous vein did not dilate after proximal compression, making visualization difficult. This affected 5 of 8 high dose animals during drug weeks 9 and 10. Beginning in week 4, 6 of 8 animals developed progressive hardening of the area along the saphenous vein. By the end of the study, cephalic, manual or pedal veins were being used since injection at the saphenous vein injection site was difficult or impossible. Absence of venous dilation was also occasionally observed in three animals in the mid and low dose groups.

ALT values were increased compared to controls at 5 mg/kg during weeks 3, (+100%), 7 (+97%), and 12 (+38%). There was a return towards normal ALT values despite continuation of dosing. AST values were 9 to 25% higher than concurrent controls in all treated dose groups and timepoints.

Injection site damage in dosed animals consisted of thickening and discoloration of veins and perivenous tissue with hemorrhage in adjacent tissue. Findings were most severe in high dose animals. In addition, venous thrombosis was detected in two males in the high dose group.

A slight subcapsular scar was seen in the liver of one high dose monkey and this was interpreted as the sequela to earlier subcapsular necrosis. Absolute and relative ovary weights were decreased in treated animals, (relative ovary weights 25, 38 and 50% less than controls at 0.5, 2 and 5 mg/kg). Also, absolute and relative prostate weights were decreased in treated animals (relative prostate weights were 40% less than controls at 5 mg/kg).

Table 5. Plasma levels of caspofungin in monkeys (24 hours postdose, µg/ml ± SEM)

Drug Week		**0.5 mg/kg**	**2 mg/kg**	**5 mg/kg**
4		0.33 (±0.02)	2.04 (±0.15)	9.2 (±0.66)
8		0.34 (±0.01)	2.08 (±0.20)	8.9 (±0.54)
12		0.43 (±0.03)	2.66(±0.22)	10.7 (±0.62)

In week 12, plasma drug levels were 26, 28 and 20 % higher than at week 8 for the 0.5, 2 and 5 mg/kg doses. As such there is clear evidence that drug accumulation continues after week 8 in monkeys (Table 5).

Table 6. Liver levels of caspofungin (µg/g liver)in monkeys after 5 and 14 weeks of caspofungin

		0.5 mg/kg	**2 mg/kg**	**5 mg/kg**
Drug Week 5				
Males			129 (±24)	316 (±33)
Females			145 (±12)	319 (±39)
Means			137	318
Drug Week 14				
Males		30.3 (±6.8)	172 (±24)	370 (± 37)
Females		34 (± 7.7)	160 (± 17)	409 (± 44)
Means		32	166	390

In week 14, liver drug levels were 21 and 23 % higher than at week 8 for the 2 and 5 mg/kg doses. As such there is clear evidence that drug accumulation continues in the liver after week 8 in monkeys (Table 6).

Absence of venous dilatation and decreased ovary weights occurred at all doses, when monkeys were treated with caspofungin for 14 weeks and so no NOAEL could be established from this study. At higher doses, caspofungin administration was associated with increased ALT levels, injection site damage (thickening and discoloration of veins and perivenous tissue with hemorrhage in adjacent tissue) and subcapsular scarring in the liver.

MRL Nonclinical report: A-10
Study Title: -Fourteen week intravenous toxicity study in rats.
Study Nos: TT#95-612-0
Document location: NDA 21-227, Vol # 5, and page # A1134:
Conducting laboratory and location: [redacted]
Date of study initiation: June 12, 1995
GLP compliance: Yes
QA- Report: Yes

Groups of Crl:CD®(SD)BR rats, 15/sex/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2 or 5 mg/kg/day for fourteen weeks. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings, histology and ophthalmic examinations (in weeks 8 and 12).

Physical signs seen in animals in the high doses could be attributed to drug-associated histamine release and included sternal recumbency, swollen snout and feet and hyperemia of the ears and feet. These signs began within 10 to 15 minutes of injection, often lasted more than six hours but were gone before the animal was ready for the next dose on the following day. These signs were not seen after day 9.

In some mid and high dose animals (beginning in week 7) tail veins did not dilate after proximal compression, making visualization and injection difficult. This affected 14 of 28 high dose, 4 of 30 mid dose, 0 of 30 low dose and 1 of 30 control animals. Eventually, three of the high dose animals could not be injected and two had to be euthanized. Purple discoloration of the tail also occurred intermittently at all doses. On microscopic examination, injection sites showed thrombosis, subcutaneous cellular infiltration, fibroplasia and hemorrhage.

AST values were slightly increased (up to + 20%) in male rats, compared to controls at all doses in weeks 7 and 12. Other parameters were similar to control values and/or were not dose dependent.

A NOAEL could be established for the administration of caspofungin to rats for fourteen weeks because increases in AST were seen at all doses. At higher doses, toxicity was characterized by an apparently histamine-mediated response, characterized by swollen/reddened ears, snouts and paws and discolored, thrombotic injection sites.

MRL Nonclinical report: A-11
Study Title: L-743872: Five week intravenous toxicity study in monkeys.
Study Nos: TT#96-073-0
Document location: NDA 21-227, Vol # 6 page A-1504
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: 19 September, 1996
GLP compliance: Yes
Drug lot number: L-743872-003M and degradates.

This study was designed to determine if the impurities generated by the degradation of caspofungin altered the toxic effects seen when administered to monkeys for five weeks. Specifically, a formulation containing a total of 4 % total degradants was tested in these animals. Groups of rhesus monkeys, *Macaca mulatta*, 4/sex/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2, or 5 mg/kg/day for five weeks. Infusion was performed via the saphenous veins of the right and left legs alternatively and the total dose was delivered in twenty minutes (0.4 to 0.6 ml/min). Dose volume was 4 ml/kg. In order to reduce the anticipated vascular reactions, venipunctures were made with a saline-filled butterfly needle and the drug infusion followed by a 5-ml manual saline flush. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings, and histology. Ophthalmic examinations were conducted on all monkeys pretest and during week 4. Plasma drug levels were measured on days 1 and 28 and liver drug levels were determined at necropsy.

ALT levels were increased by 78 and 43 % respectively at 2 and 4 weeks postdose in the high dose animals. Injection site reactions, characterized by trauma-related dermal and subcutaneous changes were similar in drug treated and control animals. One control animal had slight vasculitis and one high dose animal showed a moderate venous intimal fibrosis.
There were slight increases in the relative weights of a number of organs including the spleen (+ approximately 30 to 40%, at all doses), kidneys (+16 % at the high dose), thyroid (+ 26 % at the high dose) and the testes (up 23 % at the high dose).

Injection site reactions were less severe in this study than in previous studies, implying that the 5-ml saline flush helped to reduce the reaction. The increase in ALT is as would be expected based on the previous 5-week monkey study. The significance of the increases in organ weights is not clear, but the formulation did not contain all the degradants present in the lyophilized product.

Conclusions

Although this shows that there are no striking new toxicities produced when impurity-containing drug is used, a study needs to be conducted containing a formulation that has all the impurities that we are aware of, in relevant proportions.

MRL Nonclinical report: A-12
Study Title: Twenty seven-week intravenous toxicity study in rats.
Study Nos: TT#97-120-0
Document location: NDA 21-227, Vol # 7, and page # A1737
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: December 8, 1997
GLP compliance: Yes
QA- Report: Yes
Drug lot number:

Groups of Crl:CD®(SD)IGS BR rats, 20/sex/dose group, were treated intravenously with caspofungin at 0 (control), 1.8, 3.6, 7.2 mg/kg/day for twenty seven weeks. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings and histology. Blood was collected for toxicokinetics evaluations at 2, 4, 6, 8, 12 and 24 hours postdose in week 26.

Mortality

One low dose animal died during week 10 of urolithiasis and ascending urinary tract infection. One mid dose male died during orbital bleeding during week 24 and the death was considered to be a result of blood collection. One female was euthanized because of difficulty with dosing due to vascular irritation. One mid dose male died in week 4, after gasping and convulsions lasting 30 minutes. One high dose female died in week five after showing similar symptoms. Six high dose animals had to be sacrificed after 20 weeks because of difficulty with injections.

Physical signs seen in animals in the high doses could be attributed to drug-associated histamine release, and included sternal recumbency, (most high and 1 mid dose animals, days 1 and 2), swollen snout and/ feet (all doses, increasing in frequency as dose increased) and hyperemia of the ears and feet (mid and high doses). These signs occurred within 1 hour of dosing, but were gone before the animal was ready for the next dose on the following day. These signs were not seen after week 1.

In some (especially mid and high dose) animals there were signs of injection site irritation. Blanching of the tail, discoloration, stiffening and tail sores were likely due to caspofungin-induced vascular irritation. Elevated leukocyte counts were found in high dose males in weeks 12 and 24. Mean large unstained cell, likely reactive lymphocytes, was increased by 63 % compared to control, while mean monocyte counts were 66 and 64 % increased at 12 and 24 weeks. These increases in leukocyte counts, seen in males only, were ascribed to vascular irritation associated with repeated dosing. It is unclear why females would not show these same reactions since they show vascular irritation. Other changes seen in high dose animals included thrombosis, subcutaneous cellular infiltration, fibrosis and hemorrhage. Generalized hyperplasia of the lumbar lymph nodes is attributed to the injection site reactions and was more common in high dose animals (8/40) than controls (2/40). AST increased sporadically by a mean of about 20% in high dose rats. ALT increased by 34% in males only at week 24.

Since tail irritation and histamine-like responses were seen at all doses, a NOAEL could not be determined for caspofungin. At higher doses, caspofungin produced death, increased leukocyte counts and slight changes in liver enzymes.

MRL Nonclinical report: A-13
Study Title: Twenty seven-week intravenous toxicity study in monkey.
Study Nos: TT#97-121-0
Document location: NDA 21-227, Vol # 8, and page # A2332
Conducting laboratory and location: Merck Research Laboratories, West Point PA.
Date of study initiation: December 11, 1997
GLP compliance: Yes
QA- Report: Yes

Groups of rhesus monkeys (*Macaca mulatta)*, 4/sex/dose group, were treated intravenously with caspofungin at 0 (control), 1.5, 3.0, or 6 mg/kg/day for twenty-seven weeks. Infusion was performed via the saphenous veins of the right and left legs alternatively and delivered the total dose in twenty minutes, (1.2 to 2.5 ml/min). In order to reduce the anticipated vascular reactions, venipunctures were made with a saline-filled butterfly needle and the drug infusion was followed by a 5-ml manual saline flush. Records were kept of clinical observations, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, necropsy findings, and histology. Ophthalmic examinations were conducted on all monkeys pretest and during weeks 12 and 25. Blood was collected for possible future measurement of plasma drug levels during week 26.

Caspofungin injection in monkeys for 27 weeks produced injection site damage resulting in difficulty injecting into the saphenous vein. As a result, the brachial veins were intermittently used for injection. Treatment-related increases in ALT were seen at the highest dose and were 53, 72 and 52 % higher than controls at weeks 4, 12 and 25. Carcinoma was seen in the mammary gland of one male high dose animal. This is a very rare finding (only one other juvenile male has ever been diagnosed with this pathology). The sponsor argues that this is not related to study drug because caspofungin was not mutagenic or clastogenic in any of the genotoxicity assays. No treatment related hypertrophy or hyperplastic changes were present. No treatment related effects have been seen in shorter studies. No mammary changes have been seen in the other species tested.

Conclusion

Caspofungin administration to monkeys for 27 weeks produces changes which have been seen in studies of shorter duration, including injection site damage and liver enzyme increases. This study also showed an incident of mammary carcinoma, a very rare event. In the absence of any evidence suggesting that caspofungin is a potential carcinogen, the finding is assumed to be unrelated to drug. The sponsor does not plan to conduct an intravenous carcinogenicity study. A SHE cell assay has been recommended to the sponsor, but the sponsor has declined to conduct this study, questioning the value of this additional testing.

MRL Nonclinical report: B-1
Study Title: Intravenous fertility study in male rats.
Study Nos: TT#97-714-0
Document location: NDA 21-227, Vol # 12 page B-15
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 7 April 1997
GLP compliance: Yes
Drug lot number: L-743872(-012P001)

Groups of male Crl:CD®(SD)BR rats, 25/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2 or 5 mg/kg/day for 28 days. Each male was then paired with one female for a maximum of 10 nights. Mating was confirmed by the daily examination of females for the presence of a copulatory plug in the vagina or sperm in the vaginal canal. The day of confirmed mating was considered gestational day 0. Males were treated prior to, during and after mating, for a total of 51 to 53 days. Males were then weighed, sacrificed and subjected to gross examination of thoracic and abdominal viscera. The left caudal epididymis was used for sperm quantitation while the testes and right caudal epididymis were used for histological examinations. Females were retained until gestational day 15-17 after which animals were sacrificed and uterine contents examined. Records were also kept of physical signs, food consumption, mortality, mating performance, fertility and embryo/fetal survival.

One male in the 5 mg/kg dose group was found dead 3.5 hours after dosing on day 1. The cause of death was undetermined but the animal showed sternal recumbency, swollen snout, and swollen extremities with discolored tail within 30 minutes of injection. Physical signs seen in animals in the two higher doses could be attributed to drug associated histamine release and included sternal recumbency, swollen snout, and swollen extremities. These signs began within 30 minutes of injection, often lasted more than six hours but were gone before the animal was ready for the next dose on the following day. These signs were not seen after day 7, even in the high dose group. There were no effects of drug on male fertility indices including mating performance, fertility, caudal epididymis weights, sperm counts, sperm motility or embryonic/fetal survival.

Conclusion
Caspofungin, at intravenous doses of up to 5 mg/kg/day for seven weeks, did not significantly affect fertility indices in male Crl:CD®(SD)BR rats.

MRL Nonclinical report: B-2
Study Title: Intravenous fertility study in female rats.
Study Nos: TT#96-735-0
Document location: NDA 21-227, Vol # 12 page B-86
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 4 November 1996
GLP compliance: Yes
Drug lot number: L-743872(-003M011)

Groups of female:CD®(SD)BR rats, 22 per dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2 or 5 mg/kg/day for 16 days. Each female was then paired with one untreated male until mating was confirmed by the daily examination of females for the presence of a copulatory plug in the vagina or sperm in the vaginal canal (for a maximum of 20 nights). The day of confirmed mating was considered gestational day 0 and females were treated through gestational day 7. On gestational days 15 through 17, dams were killed and the uterus and contents examined. Indices of fertility, including corpora lutea, implantation sites, live/dead fetuses and resorptions were recorded.

There were no deaths or early sacrifices. Dams in the mid and high dose groups showed purple discoloration of the tail and signs of histamine release including sternal recumbency, swollen snout and swollen extremities within 30 minutes of drug administration. Most signs resolved within six hours (although some animals still showed swollen extremities beyond six hours). While mating performance, pregnancy rates, number of corpora lutea per pregnant female and number of dead fetuses, did not differ significantly between the dosed groups, the number of resorptions (per dose group) and percentage resorptions (resorptions/implants, litter means) were almost twice control levels in the two higher dose groups (see Table 7).

Table 7: Effect of caspofungin on peer- implantation loss and resorptions in female rats treated with caspofungin from 16 days pre mating through day 17 of gestation.

Control	Control	0.5 mg/kg	2 mg/kg	5 mg/kg
Resorptions/implants	3.7 %	3.2 %	6.7 %	7.5 %
peri-implantation loss	3.9 %	3.6 %	6.1 %	8.1 %

Discussion

There was clearly an increase in resorptions and peri-implantation losses (number of corpora lutea minus number of implants/number of corpora lutea) at the two higher doses. The sponsor argues that these findings are not significant since they are within the range of historical control values (4.4 to 9.9 for resorptions and 1 to 13 for peri implantation loss). The fact that these increases are dose-related, and were not seen in controls or low dose animals argues against that opinion.

Conclusion

Caspofungin produces increased resorptions and peri-implantation losses in female rats treated at 2 or 5 mg/kg/day during organogenesis. These doses are equivalent to human doses of 0.3 and 0.8 mg/kg/day.

MRL Nonclinical report: C-1
Study Title: Intravenous range-finding reproduction study in female rats.
Study Nos: TT#96-730-5
Document location: NDA 21-227, Vol # 14 page C-31
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 26 August 1996
GLP compliance: No
Drug lot number: L-743872(-003M011)

Groups of pregnant female:CD®(SD)BR rats, 10/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2, or 10 mg/kg/day from gestation day 6 through lactation day 20. Each female had been paired with one untreated male until mating was confirmed by the presence of a copulatory plug in the vagina or sperm in the vaginal canal. The day of confirmed mating was considered gestational day 0. All pups were counted on postnatal day 0 and 10 and randomly selected pups from each litter (5/sex where possible) were marked. On day 3, litters were further culled to 4/sex. Records were kept of dam bodyweights, physical signs, hematology analyses, serum biochemistry findings, parturition and gestation duration, and necropsy findings. The necropsy findings were restricted to examination of the uterus and associated glands. Pups were counted on postnatal day 0 and records were kept of weights, sex, external findings and physical signs.

Two high dose females were sacrificed on lactation day 0 due to damaged/necrotic tails. One dam from the 2 mg/kg dose group was sacrificed after damage to the tail and one control dam that only had one pup was sacrificed on lactation day 3.

At 5 and 10 mg/kg, drug administration was associated with sternal recumbency, hypoactivity, piloerection, swollen/red snout and/or paws. These signs became less intense with repeated dosing. At 10 mg/kg, these changes began as soon as 90 minutes after dosing and 8/10 females were sternally recumbent for 20-25 minutes after dosing. Later animals were hypoactive with piloerection and showed increased water consumption. By day 3, drug treated animals appeared very similar to control animals. At doses of 2 mg/kg and above, purple/red discoloration of the tail was noted the first three days of dosing. These signs have been seen in other toxicology studies with caspofungin and are thought to result from histamine release. The only serum biochemical changes associated with caspofungin were increases in serum triglyceride, shown below on Table 8.

Table 8. Serum Triglyceride changes associated with caspofungin administration in pregnant rats.

Caspofungin dose (mg/kg)	% Increase in serum triglyceride over control
0.5	15
2.0	14
5.0	54
10	43

Caspofungin produces toxic effects, associated with histamine release, when administered to pregnant rats. There also was an increase in serum triglyceride levels. There were no drug-related effects on the reproductive parameters examined and no external malformations or variations were reported in pups from treated dams.

Conclusion

The sponsor concluded that caspofungin was well tolerated at doses up to 5 mg/kg and that the definitive developmental toxicity study in rats should be performed at doses of 0.5, 2.0 and 5.0 mg/kg.

MRL Nonclinical report: C-2
Study Title: Intravenous developmental toxicity study in rats.
Study Nos: TT#96-736-0
Document location: NDA 21-227, Vol # 14 page C-233
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 24 November 1996
GLP compliance: Yes
Drug lot number: L-743872(-003M011)

Groups of pregnant Sprague-Dawley rats [Crl:CD®(SD)BR], 22 per dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2, or 5 mg/kg/day from gestation day 6 through gestational day 20. Each female had been paired with one untreated male until mating was confirmed by the presence of a copulatory plug in the vagina or sperm in the vaginal canal. The day of confirmed mating was considered gestational day 0. Records were kept of dam bodyweights, physical signs, food consumption, pregnancy status and necropsy findings. Fetuses were counted, weighed, sexed, and euthanized. Pups were then subjected to external, visceral, and skeletal examinations.

At 5 mg/kg, drug administration was associated with sternal recumbency, swollen/red snout, paws and ears, but these signs became less intense with repeated dosing and were not seen after day 7. At all doses, purple/red discoloration of the tail was noted sporadically during the first five days of dosing. These signs have been seen in other toxicology studies with caspofungin and are thought to result from histamine release.

Caspofungin administration was associated with an increase in peri-implantation loss and a slight increase in resorptions at 5 mg/kg. See Table 9 below.

Table 9: Peri-implantation loss and resorptions associated with caspofungin administration.

Treatment Group	Control	0.5 mg/kg caspofungin	2.0 mg/kg caspofungin	5.0 mg/kg caspofungin
Peri-implantation loss (%)	6.8	8.1	7.2	12
Resorptions (%)	5.7	3.9	4.3	7.2

Fetal abnormalities observed with caspofungin administration included cervical ribs and incomplete ossification of the torso and skull (seeTable 10 below)

Table 10: Skeletal abnormalities in fetuses from dams treated with caspofungin.

Alteration	Control	0.5 mg/kg	2.0 mg/kg	5.0 mg/kg
Cervical ribs (n)	3	7	6	19
Cervical ribs (%)	0.9	2.0	2.2	6.9
Incomplete ossification of torso (n)	7	10	12	23
Incomplete ossification of torso (%)	2.6	3.1	3.6	7.7
Incomplete ossification of sternebra (n)	3	6	7	14
Incomplete ossification of sternebra (%)	1.1	1.9	2.2	4.4
Incomplete ossification of skull (n)	1	0	1	4
Incomplete ossification of skull (%)	0.6	0	0.57	2.6

Conclusion

Caspofungin administration was associated with increases in cervical ribs and incomplete ossification of the torso (particularly, the sternebra) at all doses. At 5 mg/kg, an additional finding was incomplete ossification of the skull. A NOAEL was not determined in this study as doses as low as 0.5 mg/kg produced skeletal abnormalities.

MRL Nonclinical report: C-3
Study Title: Intravenous developmental toxicity study in rats-Postweaning evaluation.
Study Nos: TT#96-704-0
Document location: NDA 21-227, Vol # 14 page C-334
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 12 January 1997
GLP compliance: Yes
Drug lot number: L-743872(-003M011)

Groups of pregnant female:CD®(SD)BR rats, 22/dose group, were treated intravenously with caspofungin at 0 (control), 0.5, 2, or 5 mg/kg/day from gestation day 6 through lactation day 20. Each female had been paired with one untreated male until mating was confirmed by the presence of a copulatory plug in the vagina or sperm in the vaginal canal. The day of confirmed mating was considered gestational day 0. All pups were counted on postnatal day 0, and 10 randomly selected pups from each litter (5/sex where possible) were marked. On postnatal day 3 and 21, litters were culled to 4/sex and 2/sex respectively. Records were kept of dam bodyweights, food consumption, physical signs, pregnancy status, parturition and gestation duration, and necropsy findings. The necropsy consisted of an examination of the thoracic and abdominal viscera. Among pups, records were kept of litter size, dead pups, bodyweights, sex, external findings, physical signs, developmental signs (vaginal canalization or preputial separation), and behavioral assessment. Behavioral assessment consisted of passive avoidance assessment (postnatal day 35), auditory startle habituation (postnatal day 63), open field motor activity (postnatal day 70) and ophthalmic examinations (postnatal day 48). Beginning postweaning week 8, 1 male and 1 female per litter were cohabited, and records kept of physical signs, bodyweights, observation of parturition and length of gestation. All F1 males used for mating were euthanized in postnatal week 14 and F1 females that delivered were killed within a week after delivery. The uterus of each female was examined. F2 pups were counted, weighed, sexed, and examined for external malformations and mortality noted after which they were discarded without further examination.

There were no deaths in the dosed dams but two dams were sacrificed on postpartum day 1 with no surviving pups. As predicted from the histamine releasing effects seen in the previously conducted toxicology studies, dams showed sternal recumbency (day 1 at 5 mg/kg only), swollen snout (at 2 and 5 mg/kg, dose days 1 to 8), swollen extremities (ears, paws seen in all dose groups, days 1-8, with all animals in high dose group showing this finding), and discolored tails (all dose groups, days 1-8). Mortality is shown on Table 11.

Table 11: Mortality of pups of dams treated with caspofungin.

	Control	0.5 mg/kg caspofungin	2.0 mg/kg caspofungin	5.0 mg/kg caspofungin
Dead pups (n/dam*)	2 (1,1)	4 (3,1)	3(1,2)	11 (11)
Dead pups (%)	0.6	1.4	0.9	3.6

* number of deaths associated with the relevant dams

The sponsor argues that the increased deaths in the 5 mg/kg group is an incidental finding because all the deaths were from one dam. However, all the other deaths in the other groups came from a total of two dams in each group. As such, the fact that the pup deaths came from one dam does not make the finding inconsequential. There was also an increase in pups dying between postnatal days 1 and 3 in the 5 mg/kg dose group (Table 12).

Table 12: Mortality of pups of dams treated with caspofungin (postnatal days 1-3).

	Control	0.5 mg/kg caspofungin	2.0 mg/kg caspofungin	5.0 mg/kg caspofungin
Dead pups (n)	3	1	2	6
Dead pups (%)	0.9	0.6	0.6	5.4

One F1 animal each died from the control and 0.5 mg/kg groups from undetermined causes (postnatal days 32 and 36 respectively) but the lack of dose relatedness suggests that these deaths were not drug related. One F1 male died of erythroid leukemia on postnatal day 97 with enlarged liver and spleen as well as discolored lungs and kidneys. This was considered to be an incidental finding.

There were no significant effects of caspofungin on maternal bodyweights, food consumption, gross necropsy findings (F0 dams), physical signs, bodyweights, external malformations, developmental changes (vaginal canalization or preputial separation), passive avoidance test, auditory startle habituation, open field motor activity, ophthalmic examinations, maternal bodyweight or reproductive performance in F1 offspring. Survival and pup weights were comparable in treated animals to control animals. No external malformations were associated with drug treatment in F2 pups.

Summary

Caspofungin injection is associated with an increase in dead pups on day 0 and in pup deaths between days 1 and 3 when given to pregnant female:CD®(SD)BR rats (treated intravenously with caspofungin at 5 mg/kg/day from gestation day 6 through lactation day 20).

MRL Nonclinical report: C-4
Study Title: Intravenous Range-finding study in non-pregnant rabbits.
Study Nos: TT#96-731-6
Document location: NDA 21-227, Vol # 16 page C-793
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 4 September, 1996
GLP compliance: Yes
Drug lot number: L-743872(-012P001)

Groups of non-pregnant female New Zealand White rabbits, 6/dose group, were treated intravenously with caspofungin at 0 (saline control), 0 (vehicle control), 2, 4, 8 or 12 mg/kg/day for 14 days. Records were kept of bodyweights, physical signs, hematological analyses, serum biochemical analyses, and injection site (marginal ear vein) condition.

Caspofungin administration to non-pregnant rabbits produced a reduction in bodyweight at doses of 8 and 12 mg/kg, (9 and 4 % respectively, compared to controls). Serum changes are shown on the following Table 13.

Table 13. Serum changes (relative to control) associated with caspofungin administration to rabbits

	Caspofungin dose		
Parameter	**4 mg/kg**	**8 mg/kg**	**12 mg/kg**
RBC count		+ 12%	+ 8%
Hemoglobin		+ 12%	+ 5%
Hematocrit		+ 12%	+ 6%
Platelets			+ 46%
BUN		+ 25%	+ 19%
Protein		+ 9%	+ 11%
Albumin			+ 6%
AG ratio		- 12%	- 16%
Glucose			- 10%
AST		+ 430%	+ 2300%
Alkaline phosphatase	+160%	+ 210%	+310%
Calcium		+ 5%	+ 8%
Potassium	+ 10%	+ 8%	+ 13%
Phosphorus		- 6%	- 13%
Cholesterol	+170%	+ 350%	+ 340%
Triglycerides		+ 180%	+ 320%

There was no sign of irritation at the injection sites. The sponsor interpreted the findings at 8 and 12 mg/kg (decreased bodyweights, changes in liver function parameters such as the 23-fold increase in AST) as rate-limiting and selected 2, 4 and 6 mg/kg as the doses to be used in range-finding studies in pregnant rabbits.

MRL Nonclinical report: C-5:Intravenous Range-finding study in pregnant rabbits.
Study Nos: TT#96-731-5
Document location: NDA 21-227, Vol # 16 page C-850
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 10, October, 1996
GLP compliance: Yes
Drug lot number: L-743872(-012P001)

Groups of pregnant New Zealand White rabbits, 10 per dose group, were treated intravenously with caspofungin at 0 (control), 2, 4 or 6 mg/kg/day from gestation day 7 through gestational day 20. The day of confirmed mating was considered gestational day 0. Records were kept of dam bodyweights, physical signs, food consumption, hematological analyses, biochemical analyses, pregnancy-status and necropsy findings. Fetuses were counted, weighed, sexed, subjected to external examinations and euthanized before being discarded.

Caspofungin administration to pregnant rabbits produced slight increases (8-9%) in red blood cell count, hematocrit and hemoglobin compared to control. There was also an increase in the % resorptions in drug-treated animals compared to control animals. An increase in peri-implantation loss cannot be ruled out since this can be clearly observed at 2 and 4 mg/kg, even though this increase is not obvious at 6 mg/kg (see Table 14).

Table 14: Embryotoxic effects of caspofungin in Rabbits between gestational days 6 through 20

	Saline	Vehicle	2 mg/kg	4 mg/kg	6 mg/kg
% postimplantation loss	0.9	1.9	6.4	15	8.3
% peri implantation loss*	6.3	5.1	12.8	21.8	5.3

1. Postimplantation loss includes resorptions and dead fetuses
2. * (number of corpora lutea-number of implants/number of corpora lutea)

The sponsor argues that the increase in postimplantation loss was biased by low concurrent control values compared to historical controls and one animal with one implant that was an early resorption (100 % postimplantation loss). While the data from the 4 mg/kg dose group may have been biased by the data from the one animal, the trend toward an increase in postimplantation loss is still clear from the remaining dose groups. Also, concurrent controls are a more reliable representation of environmental influences under prevailing conditions. As such, the increase in postimplantation loss is judged to be drug-related. No other significant drug-induced effects were observed.

No NOAEL could be determined for embryotoxic effects since postimplantation loss was increased at the lowest dose of 2 mg/kg/day.

MRL Nonclinical report: C-6
Study Title: Intravenous Developmental Toxicity Study in Rabbits.
Study Nos: TT#96-731-0
Document location: NDA 21-227, Vol # 16 page C-932
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 31, December, 1996
GLP compliance: Yes
Drug lot number: L-743872(-012P001)

Groups of pregnant New Zealand White rabbits, 18 per dose group, were treated intravenously with caspofungin at 0 (control), 1, 3 or 6 mg/kg/day from gestation day 7 through gestational day 20. The day of confirmed mating was considered gestational day 0. Records were kept of dam bodyweights, physical signs, food consumption, hematological analyses, biochemical analyses, pregnancy status and necropsy findings. Fetuses were counted, weighed, sexed, and subjected to external, visceral and skeletal examinations.

The sponsor concludes that the dose of 6 mg/kg is maternotoxic because of a reduction in bodyweight gain between gestational days 7 and 21. In fact, the mean bodyweight of the 6 mg/kg animals on day 21 (3834g) is only 3% less than the control value (3943g). This small change in bodyweight should not be taken to indicate maternotoxicity.

Caspofungin administration was associated with increased numbers of dead fetuses and peri-implantation losses at 4 and 6 mg/kg compared to control. Additionally, at 6 mg/kg rabbits experienced an increase in % resorptions and overall postimplantation loss (See Table 15, below).

Table 15. Developmental toxicity of caspofungin in rabbits (litter means).

		Vehicle	1 mg/kg	3 mg/kg	6 mg/kg
Dead fetuses (%)		0	0	1.1	1.1
% resorptions		2.3	1.4	1.0	6.3
% postimplantation loss		2.3	1.4	2.1	7.4
% peri implantation loss*		4.4	4.5	8.7	6.0

1. Postimplantation loss includes resorptions and dead fetuses
2. * (number of corpora lutea-number of implants/number of corpora lutea)

The administration of caspofungin to rabbits at 6 mg/kg also produced an increase in the incidence of incomplete ossification of the talus/calcaneus. There is also an increase in the incidence of reduced 13th rib at 4 and 6 mg/kg (Table 16).

Table 16. Developmental toxicity of caspofungin in rabbits (litter means).

		Vehicle	1 mg/kg	3 mg/kg	6 mg/kg
Reduced 13th rib		15	17	25	19
Incomplete ossification of talus/calcaneus		6	0	4	12

Conclusions

The administration of caspofungin to rabbits at 3 and 6 mg/kg produced increased peri and postimplantation losses as well as bone abnormalities such as incomplete ossification of the talus/calcaneus and reduced 13th rib. The NOAEL for embryotoxic effects was 1 mg/kg/day.

MRL Nonclinical report: C-7
Study Title: Intravenous Toxicokinetics Study in Pregnant Rabbits.
Study Nos: TT#97-719-0
Document location: NDA 21-227, Vol # 16 page C-1009
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486,

Date of study initiation: 3 June, 1997
GLP compliance: Yes
Drug lot number: L-743872(-003M-0111)

A group of 10 pregnant female New Zealand White rabbits were given a daily intravenous dose of 5 mg/kg caspofungin from gestational day 7 through 20. On day 20, blood was sampled from dams and fetuses and assessed for caspofungin pharmacokinetics parameters. Blood was sampled at 2, 4, 8 and 24 hours post-dosing and each animal was bled twice. Results are shown in Table 17.

Table 17 Maternal and fetal concentrations of caspofungin on GD 20 following intravenous administration of 5 mg/kg/day.

Time postdose (h)	Maternal µg/ml	Fetal µg/ml	Fetal/maternal
2	23		
4	13.7	0.71	0.05
8	12.8		
24	1.49	0.43	0.29
Cmax µg/ml	23		
AUC(2-24) µg*hr/ml	203		

Conclusion

Fetuses carried by rabbit dams treated with caspofungin at 5 mg/kg/day are exposed to the drug at levels representing 5 and 29% of the corresponding maternal values.

MRL Nonclinical report: C-8
Study Title: Intravenous Toxicokinetics Study in Pregnant and lactating rats.
Study Nos: TT#97-718-0
Document location: NDA 21-227, Vol # 16 page C-1053
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486,

Date of study initiation: 29 May 1997
GLP compliance: Yes
Drug lot number: L-743872(-003M-0111)

Groups of Sprague Dawley rats were treated with caspofungin at 5 mg/kg/day in order to assess the toxicokinetics of this drug in pregnant and lactating rats. In the first part of the

experiment (the gestation phase), a group of 8 pregnant rats was treated between gestational days 6 through 20 and plasma drug levels measured in dams and pups on day 20 at 4 and 24 hours postdose. In the second (lactation phase), a group of 4 lactating rats were treated with caspofungin from lactation day 1 through lactation day 14. Plasma and milk levels of caspofungin were assessed on lactation day 14 at 4 hours postdose. The results are shown below in Tables 18 and 19.

Table 18: Maternal and fetal plasma concentrations of caspofungin on gestational day 20

Time postdose (h)	Maternal (μg/ml)	Fetal (μg/ml)	Fetal/maternal
4	17.8	0.62	0.03
24	1.78	0.32	0.18

Table 19: Maternal plasma and milk concentrations of caspofungin on lactation day 14

Time postdose (h)	**Maternal (μg/ml)**	**Fetal (μg/ml)**
4	19.5	2.6

The mean fetal plasma concentrations of caspofungin on gestational day 20 are about 3 and 18% of the maternal values, while the amount in milk on lactation day 14 was about 13% of the maternal plasma levels on that day. These findings in rats suggest that caspofungin is likely to be transferred from mother to child if taken by pregnant or nursing mothers.

MRL Nonclinical report: D-1
Study Title: Microbial Mutagenesis Assay
Study Nos: TT#94-8053, TT#94-8054, TT#95-8000 and TT-8009
Document location: NDA 21-227, Vol # 18 page D-25
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486,

Date of study initiation: TT#94-8053: 11 November, 1994
TT#94-8054: 28 November, 1994
TT#95-8000: 19 December, 1995
TT#95-8009: 13 February, 1995

GLP compliance: Yes
Drug lot number: L-743872-003M006)

Study Endpoint: Two-fold increase in revertants

These studies were designed to determine if caspofungin induced mutations, measured as reversion to histidine prototrophy of specific histidine auxotrophic strains of *Salmonella typhimurium* and as reversion to tryptophan prototrophy of specific tryptophan strains of *Escherichia coli*. *Salmonella* strains tested were TA1535, TA97a, TA98 and TA100. *E. coli* Strains tested were WP2, WP2 uvrA, WP2 uvrA pKM101.

The basis of dose selection was a preliminary microbial mutagenicity assay, in which caspofungin, at doses up to 1000 µg/plate did not induce a two-fold increase in revertants relative to solvent controls. The positive control compound, 2-aminoanthracene (2-AA), produced the expected increases in most tested species except TA-1535.

Comment

The maximum dose used in this preliminary assay was 1000 µg/plate, selected "because of the limited availability of the material and because of toxicity seen in the past with similar compounds" according to the sponsor. Although the sponsor increased the dose 10-fold for the definitive assay (to 10,000 µg/plate), the basis for the selection of this dose was unacceptable.

Doses tested were between 1, 3, 10, 30, 100, 300, 600, 1000, 2000, 3000 and 10,000 µg/plate with and without metabolic activation. Positive controls were 2-aminoanthracene (2-AA), with or without metabolic activation except *E. coli* WP2, for which the control was [redacted] Negative control was vehicle (DMSO). Liver homogenates (S-9) were prepared from rats treated with Phenobarbital/beta-naphthoflavone. Half of a ml of the activation system or buffered saline, was added to the various concentrations of caspofungin or controls, followed by 2 ml of soft agar containing 0.1 ml of the suspension of the organism under study. The mixture was gently agitated and poured onto a base layer of agar. Triplicate plates containing only trace amounts of histidine or tryptophan and one supplemental plate with sufficient histidine and tryptophan for growth of auxotrophs were prepared for each treatment group with or without metabolic activation. After incubation for 48 hours at 37 degrees C, revertant colonies on the histidine- or tryptophan-deficient plates were counted and the supplemental plates examined for evidence of inhibition or contamination. Revertant colony counts were averaged and compared with the appropriate control plate values.

In 94-8054, caspofungin did not produce any two-fold increases in revertant number relative to control. The direct or indirect acting controls gave the expected increases in revertants in this test. Inhibition of background lawn growth was seen at doses as low as 300 µg/plate in some tester strains. Inhibition of revertant growth was noted in all tester strains at the highest doses, but was most severe in TA97a and WP2uvrA, so that a repeat assay was performed with these two strains over a smaller range of doses. The repeat study 94-8054 confirmed that caspofungin did not induce a two-fold increase in revertants relative to controls at the concentrations tested. To confirm these negative results, the assay was repeated using doses designed to minimize interference in the assay (Study 95-8000). Again, caspofungin did not increase the number of revertants and all controls produced the expected responses.

Chemical assay of the solutions indicated that not all concentrations were within acceptable limits. The stability of caspofungin in DMSO at low concentrations was not adequate. In study 95-8000 most doses were below specification but inhibition of background lawn or revertant growth was seen for all Salmonella strains, leading the sponsor to the conclusion that the desired high dose had been achieved (dose at which biological effects had been observed). In the *E. coli* strains, only minimal biological effects were seen, invalidating this portion of the test

and necessitating a repeat assay with these strains (study 95-8009). In this repeat study, caspofungin did not induce increases in revertant numbers relative to control in *E. coli*.

MRL Nonclinical report: D-2
Study Title: Microbial Mutagenesis Assay
Study Nos: TT#96-8078, TT#96-8080, TT#95-8003
Document location: NDA 21-227, Vol # 18 page D-91
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, [redacted]
Date of study initiation: TT#94-8078: 12 December, 1996
TT#94-8080: 13 December, 1996
TT#95-8003: 5 February, 1997
GLP compliance: Yes
Drug lot number: L-743872-012P001
Study Endpoint: Two-fold increase in revertants

These studies were designed to determine if caspofungin induced mutations, measured as reversion to histidine prototrophy of specific histidine auxotrophic strains of *Salmonella typhimurium* and as reversion to tryptophan prototrophy of specific tryptophan auxotrophic strains of *Escherichia coli*. *Salmonella* strains tested were TA1535, TA97a, TA98 and TA100. *E. coli* Strains tested were WP2 B/r, WP2 uvrA, WP2 uvrA pKM101.

The basis of dose selection were a number of microbial mutagenicity assays in which caspofungin, at doses up to 10,000 µg/plate did not induce a two-fold increase in revertants compared to solvent controls. The studies indicated different sensitivities among the bacterial test strains and so plating was performed on two separate days. Salmonella strains were run on one day (study #96-8080), and *E. coli* strains run on another (study #96-8078). The positive control compound, 2-aminoanthracene (2-AA), produced the expected increases in all tested species.

Doses tested were between 1 and 10,000 µg/plate with and without metabolic activation. Positive controls were 2-aminoanthracene (2-AA), with or without metabolic activation except *E. coli*. WP2, for which the control was [redacted] Negative control was vehicle (DMSO). Liver homogenates (S-9) were prepared from rats treated with Phenobarbital/beta-naphthoflavone.

In 96-8080, caspofungin did not produce any two-fold increases in revertant number relative to control. The direct or indirect acting controls gave the expected increases in revertants in this test. Chemical assays showed that many concentrations were lower than expected, but that in all instances, except for TA1535, the highest testable dose had been achieved, as evidenced by decreased revertant growth. As such, TA1535 was retested in study 97-8003. In 96-8078, there was also no increase in revertants in the tested species, but TA97a showed an unusually low revertant number and so the assay was repeated with this strain (also in study 97-8003). In study 97-8003, again, caspofungin did not increase the number of revertants in the tested strains and all controls produced the expected increases in revertants.

MRL Nonclinical report: D-3
Study Title: In vitro Alkaline elution/Rat hepatocyte Assay.
Study Nos: TT#94-8244 and TT#94-8245
Document location: NDA 21-227, Vol # 18 page D-177
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486,

Date of study initiation: TT#94-8244: 20 October, 1994
TT#94-8245: 25 October, 1994

GLP compliance: Yes
Drug lot number: L-743872-012P001
Study Endpoint: An induced elution slope greater than or equal to 0.034, not associated with any cytotoxicity.

This study was designed to determine if caspofungin is associated with DNA strand breaks without cytotoxicity in primary rat hepatocytes dosed in vitro. For each drug concentration (5-42 μm), one 5 ml aliquot of cell suspension was taken from each of two separately dosed plates and each loaded onto a

Conclusion

Caspofungin did not cause any DNA strand breaks at 5-42 μM in the In vitro Alkaline elution/Rat hepatocyte Assay.

MRL Nonclinical report: D-4
Study Title: In vitro Alkaline elution/Rat hepatocyte Assay.
Study Nos: TT#96-8313
Document location: NDA 21-227, Vol # 18 page D-223
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: November 19, 1996
GLP compliance: Yes
Drug lot number: L-743872-012P001
Study Endpoint: An induced elution slope greater than or equal to 0.02, not associated with any cytotoxicity.

This study was designed to determine if caspofungin is associated with DNA strand breaks without cytotoxicity in primary rat hepatocytes dosed in vitro [at concentrations (9-42 μM)].

[redacted]

Conclusion

Caspofungin did not cause any DNA strand breaks at 5-42 μM in the In vitro Alkaline elution/Rat hepatocyte Assay.

MLR Nonclinical report: D-5
Study Title: Assay for chromosomal aberrations *in vitro* in Chinese hamster ovary cells
Study Nos: TT#96-8677 and TT#94-8791
Document location: NDA 21-227, Vol # 18 page D-282
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: October 25, 1994
GLP compliance: Yes
Drug lot number: L-743872-003M006
Study Endpoint: An increase in the percentage of cells with chromosomal aberrations at two separate concentrations.

The objective of this study is to determine if caspofungin induced chromosomal aberrations in the Chinese hamster ovary (CHO) cell line. Caspofungin was dissolved in DMSO and tested at doses between 1 and 100 μM. Assays were conducted with and without S9 and positive controls were cyclophosphamide and mitomycin C. Dose selection was based on the preliminary solubility test (TT#94-8791), when caspofungin was reconstituted in McCoy's 5A medium and precipitate was observed at 85μM, (at the end of a three-hour incubation). Since precipitates can interfere with scoring, the high dose was chosen to be 100μM.

Cultures were treated for three hours, washed twice, re-fed with complete medium, and re-incubated at 37degrees C for a further 17 hours, then harvested. All flasks received 0.1μg/ml colcemid to induce mitotic arrest two to three hours before harvest. Cultures were inspected before harvest and cytotoxicity recorded. Twenty-four hours after the beginning of treatment, the cultures were harvested by trypsinization. An aliquot of cells from each flask was counted by Coulter counter and the total number of cells in each flask was calculated and expressed as a percentage of the controls. The remaining cell suspension was fixed and slides were prepared and stained with Giemsa for analysis of chromosomal aberrations.

There were no statistically significant increases in the percentage of cells with chromosomal aberrations at any of the caspofungin doses tested. At the higher doses some precipitation was seen and if it was barely discernable, aberrations were scored. Where precipitate was clearly visible, the incubate was not included in the analysis. The positive control compounds induced significant increases in aberrations over controls, indicating that the assay was working as expected.

Conclusion

Caspofungin was negative in the in vitro assay for chromosomal aberrations in CHO cells with or without S-9.

MLR Nonclinical report:D-6
Study Title: Assay for chromosomal aberrations *in vitro* in Chinese hamster ovary cells.
Study Nos: TT#96-8724 and TT#96-8732
Document Location: NDA 21-227, Vol #18 page D-354
Conducting laboratory and location: Merck Research Laboratories, West Point PA 19486 USA
Date of study initiation: TT#96-8724 (7, November 1996) and TT#96-8732 14 November 1996.
GLP compliance: Yes
Drug lot number:L-743872-012P-001
Study Endpoint: An increase in the percentages of cells with chromosomal aberrations at two separate concentrations.

The objective of this study is to determine if caspofungin (lyophilized formulation, with degradants) induced chromosomal aberrations in the Chinese hamster ovary cell line. Caspofungin was dissolved in saline and tested at doses between 1 and 80μM

The top doses scored for chromosome aberrations were limited by the presence of precipitate. There were no statistically significant increases in the percentage of cells with chromosomal aberrations at any of the caspofungin doses tested. The positive control compounds induced significant increases in aberrations over controls, indicating that the assay was working as expected.

Conclusion

Caspofungin (lyophilized formulation, with degradants) was negative in the *in vitro* assay for chromosomal aberrations in CHO cells with or without S-9.

MLR Nonclinical report: D-7
Study Title: V-79 Mammalian Cell Mutagenesis Assay.
Study Nos: TT#96-8502 and TT#96-8504
Document location: NDA 21-227, Vol # 18 page D-419
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: TT#96-8502, 18 January 1996 and TT#96-8504, 12 February 1996
GLP compliance: Yes
Drug lot number: L-743872-003M001
Study Endpoint: Statistically significant increase in induced mutant fraction.

This study was designed to determine if caspofungin induces mutation, measured as resistance to 6-thioguanine, at the *hpt* locus in V-79 Chinese Hamster lung cells

Doses selected (0.015 to 0.055 mM) were based on solubility seen with caspofungin in range finding studies, in which precipitate was seen at the highest dose tested, (0.06 mM). Vehicle was DMSO, as was the negative control, positive controls were [redacted] with S-9 and [redacted] without S-9. Cultures in T150 flasks containing $9.84x10^6$ cells (TT#96-8502) or $1.57 x10^7$ (TT#96-8504) were exposed to treatment on day 0. All flasks were subcultured during an expression period of nine days and the cells were dispensed at $3x10^5$ cells per 100 mm petri plate in 11 µg/ml 6-thioguanine for selection. Plating efficiency at selection was determined by seeding a total of 450 cells into three 100 mm petri plates without the addition of [redacted]

Caspofungin did not induce mutation in V-79 cells at the *hpt* locus under testing conditions, with or without metabolic activation. The positive controls, [redacted] both produced significant increases in mutations, validating the assay.

MLR Nonclinical report: D-8
Study Title: Assay for chromosomal aberrations in mouse bone marrow.
Study Nos: TT#95-8713
Document location: NDA 21-227, Vol # 18 page D-483
Conducting laboratory and location: Merck Research Laboratories, West Point PA, 19486, USA
Date of study initiation: 17 October 1995
GLP compliance: Yes
Drug lot number: L-743872-003M011
Study Endpoint: Statistically significant increase in the percentage of cells with chromosomal aberrations at two dose points

The objective of this study was to determine if caspofungin induced chromosome aberrations in the bone marrow cells of female Crl:CD-1®(ICR)BR mice.

High doses selected, 12.5 mg/kg was on preliminary studies which showed that higher doses (25 mg/kg and above) caused death in these mice. Vehicle was 0.9 % saline, as was the negative control, positive control was mitomycin C.

Animals were treated with caspofungin or negative control in a single dose via the tail vein. The positive control was injected intraperitoneally. Positive control animals were sacrificed at 24 hours postdose. Caspofungin and negative control animals were sacrificed at 6, 24 and 48 hours.

All animals received 2 mg/kg colchicine, intraperitoneally, about 3 hours prior to sacrifice. Animals were sacrificed by cervical dislocation, both femurs quickly removed and crushed, and bone marrow harvested in warm Hank's Balanced salt solution. Bone marrow cells were treated with hypotonic solution of 0.5 % potassium chloride in water, and fixed in absolute methanol:glacial acetic acid (3:1). Fixed cells were then placed on slides, coded to correlate to animal number, and stained with Giemsa. Slide analysis was performed from 50 cells per mouse where possible.

Caspofungin did not induce chromosome aberrations in the bone marrow cells of female Crl:CD-1®(ICR)BR mice. Positive controls caused the expected responses, validating the performance of the assay.

Conclusion

Caspofungin did not induce chromosome aberrations in the bone marrow cells of female Crl:CD-1®(ICR)BR mice.

Carcinogenesis

No long-term studies in animals were performed to evaluate the carcinogenic potential of caspofungin.

Summary and Conclusions

Acute Studies

Acute toxicity studies conducted in mice, rats and rabbits, showed that the minimum lethal dose of caspofungin ranged from 2-8.4 mg/kg (equivalent human doses, based on body surface area conversions). The maximum nonlethal doses ranged from 1 mg/kg (mice) to 3.9 in rabbits and 4.2 in rats. Mice were four times more sensitive to caspofungin than rats and rabbits, and were not used for the repeat dose studies. Tremors, clonic convulsions, decreased activity, sternal recumbency and bradypnea accompanied deaths in these acute studies.

Repeat dose studies

Repeat dose studies were conducted in rats and monkeys for general toxicology and in rabbits to assess reproductive toxicity. The most common findings were allergic-type reactions that are characteristically signs of histamine release, injection site damage/thrombosis and liver toxicity.

In rats, but not monkeys, caspofungin injection was associated with signs of histamine release. These included hyperemia of the ears and feet, swelling of snout and feet, bradypnea, decreased activity and sternal recumbency. These signs occurred within one hour of dosing, could last for six hours or more, but were usually gone before dosing the following day. Liver toxicity was seen in rats, rabbits and monkeys, and included increases in liver enzymes (AST, ALT, alkaline phosphatase) and liver necrosis. Injection site damage was seen in all species and consisted of purple/red discoloration, failure of veins to dilate after proximal compression, thrombosis, subcutaneous cellular infiltration, fibroplasia and hemorrhage.

Reproductive toxicology studies

Injection of caspofungin into pregnant/lactating rats showed that caspofungin passed from the mother to the fetus and to the milk. Adverse events associated with caspofungin administration to rats and rabbits included incomplete ossification of the skull, torso and talus, as well as increased resorptions, peri-implantation losses and dead fetuses/pups. There was also an increase in the incidence of cervical rib.

Carcinogenesis, Mutagenesis and Impairment of Fertility

Carcinogenicity studies were not performed with caspofungin. Caspofungin did not show any evidence of mutagenicity or impairment of fertility.

Labeling

The label will contain the following information

Carcinogenesis, Mutagenesis, and Impairment of Fertility

No long-term studies in animals have been performed to evaluate the carcinogenic potential of caspofungin.

Caspofungin did not show evidence of mutagenic or genotoxic potential when evaluated in the following *in vitro* assays: Bacterial (Ames) and mammalian cell (V79 Chinese Hamster lung fibroblasts) mutagenesis assays, the alkaline elution/rat hepatocyte DNA strand break test, and the chromosome aberration assay in Chinese hamster ovary cells. Caspofungin was also not genotoxic when assessed in the mouse bone marrow chromosomal test at doses up to 12.5 mg/kg (equivalent to a human dose of 1 mg/kg based on body surface area comparisons), administered intravenously.

Fertility and reproductive performance were not affected by the intravenous administration of caspofungin to rats at doses [redacted] EXPOSURES SIMILAR TO THOSE SEEN IN PATIENTS TREATED AT THE 70 MG DOSE.

Pregnancy

Pregnancy Category C. CANCIDAS has been shown to be embryotoxic in rats and rabbits. Findings included incomplete ossification of the skull, talus/calcaneus, and torso, [redacted] cervical rib. Caspofungin also [redacted] There are no adequate and well-controlled studies in pregnant women. CANCIDAS should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether caspofungin is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when [redacted] is administered to nursing women.

Conclusion

In summary, there are no data that would preclude the approval of caspofungin for the treatment of patients with invasive aspergillosis which have failed standard therapy.

/S/

Owen G. McMaster, Ph.D.
Pharmacology/Toxicology Reviewer, DSPIDP

Concurrences:
HFD-590/DeputyDirector /S/ 1/24/01
HFD-590/Pharm/ToxTL /S/ 1/24/01

Disk:
HFD-590/HastingsK
cc:
HFD-590 Original IND
HFD-590/Biopharm/HigginsK
HFD-590/BiopharmTL/AjayiF
HFD-590/PM/ChanL
HFD-590/Chem/MatekaD
HFD-590/Chem/HolbertG
HFD-590/ChemTL/SchmuffN
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